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Studying the Effects of Supplementing (Reashure) to Pregnant Sheep on Incidence of Ketosis and Health Status Pre and after Lambing

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Abstract

Pregnancy toxemia is a metabolic disease of pregnant ewes which causes significant economic losses in the sheep industry due to maternal and fetal death. Early and accurate diagnosis of subclinical metabolic disorders, like pregnancy toxemia and ketosis, is important for the dairy sheep industry. The main objective of the present study is to increase understanding (awareness) about pregnancy toxemia in sheep. Specific objectives are:

- To evaluate blood ketone and glucose levels as screening tests for the detection of pregnancy toxemia in sheep (ketosis)
- To study the impact of feeding protected choline (ReaShure) on the incidence of pregnancy toxemia during pre and postpartum period

BHB level and glucose level tests have recently become increasingly available and could be used as simple, rapid tests for both the diagnosis of sick sheep and the routine monitoring of herd group. Blood glucose measurement is not a precise and reliable index for evaluation of SCK in sheep. Feeding protected choline (ReaShure) to sheep during pregnancy reduces incidence of pregnancy toxemia (ketosis), abortion and animals were healthier.

Keywords: Pregnancy toxaemia-Ketosis; Beta-hydroxybutyrate (BHB); Hypoglycemic; Hyperglycemic; Hyperketonemia; Propylene glycol; Protected choline (ReaShure); Acetone or Acetoacetate (AcAc); BHB check plus (Blood ketone and Glucose test system); Qucare vet meter; Qucare vet strips; Subclinical ketosis-abortion

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Introduction

Pregnancy toxemia is a metabolic disorder in sheep and goats which usually develops during late gestation and which is always associated with hyperketonemia and hypoglycemia. Ewes of certain breeds, mainly when bearing two or three lambs, are more susceptible than ewe's only one fetus [1]. The economic significance of the disease is determined by reduced milk yields and body weight loss, poor feed conversion, increased culling and mortality rates of offspring and affected animals [2].

Compared to dry ewes, ewes in late pregnancy require about 50% more feed if bearing a single lamb and about 75% more feed if carrying twins. This amount of feed may exceed their intake capacity unless the grain is substituted for part of the ration [3]. Ovine pregnancy toxemia or "twin-lamb" disease occurs in the latter part of pregnancy in sheep, typically in ewes with multiple fetuses, and is characterized by anorexia and neurologic signs of motor weakness, amaurosis, and mental dullness. It may occur spontaneously or may be induced by dietary deficiency. The disease is associated with the high calorific requirements of pregnant sheep. Pregnancy toxemia may occur following a relative deficiency of available carbohydrate that leads to a drain on oxaloacetate and its precursors in an effort to maintain blood glucose concentration. Toxemia can usually be prevented by adequate supplementary feeding in late pregnancy. Clinical pathologic studies show consistent elevation of blood ketones and glucocorticoids and, in the early part of the disease, decreased blood glucose. Subclinical ketosis is defined as elevated concentration of circulating ketone bodies in the absence of clinical signs [4]. There are four categories of disease according

to Edmondson et al. [5]: 1) Primary pregnancy toxemia caused by inadequate nutrition (poor quality feed, period of fasting); 2) Fat ewe/doe pregnancy toxemia seen in over-conditioned ewes/does in early gestation (suffer a nutritional decline in late gestation, possibly from smaller rumen capacity); 3) Starvation pregnancy toxemia seen in severely under-conditioned ewes/does (lack of feed after drought, heavy snow or flood) and; 4) Secondary pregnancy toxemia due to concurrent disease such as parasites, poor dentition, or lameness. Hyperketonemia is defined as an abnormally high concentration of circulating ketone bodies during the postpartum period. The gold standard diagnostic test for hyperketonemia is the measurement of hydroxyl butyric acid (BHBA) in serum or plasma by a laboratory process [6]. The most commonly used level of serum BHB to identify ketosis is a concentration ≥ 1400 mmol/l (14.4 mg/dl) [7]. An alternative to the laboratory method is blood BHBA measurement that can be used as on-farm cow side test for hyperketonemia with a near-perfect accuracy (Precision Xtra; sensitivity 96%, specificity 97%).

More recently, cow side tests for ketosis have focused on measuring BHBA level. One test is Keto Test (Sanwa Kagaku Kenkyusho Co Ltd, Nagoya, Japan; distributed by ELANCO Animal Health, Greenfield, IN). This test measures BHBA in milk and consists of test strips on which a reagent converts BHBA in the milk sample to AcAc. Using a provided color scale, the strip may be used to semi-quantitatively measure BHBA concentration s in the milk sample based on intensity of the color change observed on test strip [8,9]. A new blood strip test for BHBA and glucose was developed and manufactured by DFI CO., Ltd (South Korea). Recently a new test BHB Check Plus (blood ketone and glucose test system) manufactured by PortaCheck, Inc, USA.

Laboratory findings in individual animals may include hypoglycemia (often <2 mmol/l), elevated urine ketone levels, elevated BHBA levels (normal <0.8 mmol/l, subclinical ketosis >0.8 mmol/l and clinical disease >3.0 mmol/l) and frequently hypocalcemia and hyperkalemia due to severe ketoacidosis [10]. Blood glucose concentration between 40 and 65 mg/dl are common in pregnancy toxemia, although comatose animals may show terminal hyperglycemia, especially associated with fetal death [11].

To prevent ketosis in sheep, goats, it is important to identify the animals carrying twins or triple, separate them and provide them with a diet that will meet their energy demand. Successful treatment of pregnancy toxemia requires early detection and steps to quickly meet the energy (glucose) needs of the affected ewe. The most common treatment is to drench ewes with 2 to 3 ounces of propylene glycol 2 to 3 times daily. Propylene glycol, which is mainly absorbed intact directly from the rumen at a rate of 40% per hour and reaches its maximum blood level within 30 min of administration and maximum blood glucose conversion at about 4 hrs. after administration. Propylene glycol transformation in glucose probably occurs via conversion to pyruvate [12].

ReaShure microencapsulated choline, a recent technological breakthrough, protects choline on its journey rough- and -tumble rumen and releases it in the small intestine. Lipid (fat) layers, applied using a proprietary process, encapsulate (coat) the choline. At the time of calving and during negative energy balance, feeding ReaShure increases fat export out of the liver which prevents fatty liver and reduces the amount of fatty acids converted to ketones by the liver [13,14]. Feeding ReaShure also reduced the incidence of mastitis (p=0.06) and all postpartum diseases combined (p=0.001). Clearly, cows fed ReaShure were healthier and produced more milk [15].

Material and Methods

Two groups of sheep around calving (with history of twin pregnancy), Nagdi breed.

- Treated Group: 35 sheep, ReaShure will be administered 30 days before lambing and 30 days after lambing at a dosage of 10 g/head/day
- Control Group: 34 sheep, No ReaShure

Feeding ration

The feeding is consisting of 50% Alfa-Alfa (1125 kg for 45 days) and 50% Rhodes Grass (1125 kg also for 45 days), plus 2250 kg of pellets concentrates. The feeding rate is sheep/day is 2 kg/day (1 Kg morning and 1 kg in the evening). A source of Glucose supplements is given to the control group in water as an energy source (Glycerine).

A test group is given ReaShure at 10 g/head/day for 45 days (25 kg bag). The total quantity is mixed thoroughly with alfa-alfa and Rhodes then packed in 25 kg bags for feeding.

The aim is to study the impact of feeding (ReaShure) on the incidence of pregnancy toxemia during pre and postpartum period by monitoring and evaluation of the following parameter:

1. Ketosis incidence of 10-15 days pre-lambing in both groups
2. Ketosis incidence 7-10 days after lambing in both groups
3. Abortion incidence of pre-lambing
4. Sheep mortality incidence

Laboratory findings in pregnancy toxemia ewes include elevated BHB levels, (normal <0.8 mmol/l, subclinical ketosis >0.8 mmol/L and clinical disease >3.0 mmol/l). Normal Blood glucose level is between 40 and 65 mg/dl, <40 mg/dl is hypoglycemic, >70 mg/dl is hyperglycemic.

Blood sampling

Blood samples were taken from both control and test group 10-15 days pre-lambing and 7-10 days after lambing. Blood samples were tested for BHB level and glucose level using BHB Check Plus (Blood Ketone and Glucose test system) from PortaCheck, USA and also Qucare Vet Meter with Qucare vet strips from DFI South Korea. Abortion incidence as well mortality incidence was recorded.

Results

Pre-lambing period results in the control group

Glucose results: Normal Glucose level is 40-65 mg/dl. Animals with normal glucose levels are 16 out of 34, hypoglycemic is 0 and hyperglycemic are 18 **Tables 1 and 2**.

Table 1: BHB/Glucose measurements, control group.

Before Lambing					After Lambing				
Animal No	Date	BHB mmo/l	Glucose mg/dl	Date	BHB mmol/l	Glucose mg/dl	BHB reading difference	Glucose reading Difference	Comments
50116	28-Jul	0.8	high	24-Aug	0.9	61	Increased	Decreased	
35766	28-Jul	1.1	high	24-Aug	0.5	57	Improved	Decreased	
50951	28-Jul	0.7	high	24-Aug	0.4	61	Improved	Decreased	
51011	28-Jul	0.9	high	24-Aug	Died	Died	Died	Died	
30947	28-Jul	1	high	24-Aug	0.6	73	Improved	Increased	Hyperglycaemic
37905	28-Jul	0.5	High	24-Aug	0.9	37	Increased	Decreased	Hypoglycaemic
29883	28-Jul	0.6	51	24-Aug	0.5	63	Improved	Increased	
52539	28-Jul	0.7	64	24-Aug	0.5	71	Improved	Increased	Hyperglycaemic
38214	28-Jul	0.5	53	24-Aug	0.4	57	Improved	Increased	
29975	28-Jul	0.7	56	24-Aug	0.9	53	Increased	Decreased	
29461	28-Jul	0.8	high	24-Aug	0.5	57	Improved	Decreased	
366779	28-Jul	0.4	high	24-Aug	0.5	79	Increased	Increased	Hyperglycaemic
51188	28-Jul	1.2	high	24-Aug	0.6	70	Improved	Decreased	
41930	28-Jul	0.5	40	24-Aug	0.4	71	Improved	Increased	Hyperglycaemic
52552	28-Jul	0.6	51	24-Aug	0.4	79	Improved	Increased	Hyperglycaemic
41603	28-Jul	0.6	67	24-Aug	0.5	73	Improved	Increased	Hyperglycaemic
25249	28-Jul	0.5	57	24-Aug	0.5	62	No change	Increased	
37867	28-Jul	1.1	55	24-Aug	5.9	64	Increased	Increased	
41587	28-Jul	0.4	68	24-Aug	0.4	70	No change	Increased	
51902	28-Jul	0.5	65	24-Aug	0.6	73	No Change	Increased	Hyperglycaemic
52525	28-Jul	0.6	71	24-Aug	0.5	79	Improved	Increased	Hyperglycaemic
30192	28-Jul	1.2	47	24-Aug	0.4	71	Improved	Increased	Hyperglycaemic
38270	28-Jul	1.4	70	24-Aug	0.5	66	Improved	Decreased	
51317	28-Jul	0.6	high	24-Aug	0.5	78	Improved	Increased	Hyperglycaemic
36422	28-Jul	0.5	high	24-Aug	3.4	41	Increased	Decreased	
51219	28-Jul	0.8	48	24-Aug	0.6	63	Improved	Increased	
50235	28-Jul	1.3	high	24-Aug	Died	Died	Died	Died	
40663	28-Jul	2.2	high	24-Aug	0.7	61	Improved	Decreased	
30114	28-Jul	0.7	high	24-Aug	0.7	63	No change	Decreased	
28253	28-Jul	1.3	64	24-Aug	0.5	73	Improved	Increased	Hyperglycaemic
36385	28-Jul	0.5	high	24-Aug	0.5	84	No change	Increased	Hyperglycaemic
35734	28-Jul	0.6	62	24-Aug	1	50	Increased	Decrease	
29737	28-Jul	1.1	high	24-Aug	1.5	72	Increased	Increased	Hyperglycaemic
52598	28-Jul	0.8	high	24-Aug	0.5	86	Improved	Increased	Hyperglycaemic
Normal Range		Normal ≤ 0.8 mmol/l	Normal 40-65 mg/dl		Normal ≤ 0.8 mmol/l	Normal 40-65 mg/ dl			
		Subclinical >0.8 mmol/l	Hypoglycemic, <40 mg/dl		Subclinical>0.8 mmol/l	Hypoglycemic, <40 mg/dl			
		Clinical>3 mmol/l	Hyperglycemic, >70 mg/dl		Clinical>3 mmol/l	Hyperglycemic,>70 mg/dl			

Table 2: Glucose level.

Total Animal	Normal	Hypoglycemic	Hyperglycemic
34	16	-	18
%	47.06%	0%	52.94

Table 3: BHB Level.

Total Animal	Normal	Subclinical Ketosis	Clinical Ketosis
34	23	11	0
%	67.65%	32.35%	0%

Biochemical results and health status pre-lambing summary (Control group).

BHB level results: normal <0.8 mmol/l, subclinical ketosis >0.8 mmol/l and clinical ketosis >3.0 mmol/l. Animals with normal BHB are 23 out of 34 and those with subclinical ketosis are 11 and 0 with clinical ketosis **Tables 1 and 3**.

After lambing period results in the control group

Glucose results: Animals with normal glucose levels are 17, 1 is hypoglycemic and 14 were hyperglycemic **Tables 1 and 4**.

Biochemical results and health status after-lambing summary (Control group) are given below.

Table 4: Glucose Level.

Total Animal	Normal	Hypoglycemic	Hyperglycemic
32	17	1	14
%	53.12%	3.13%	43.75%

BHB level: 25 animals are with normal BHB, 5 are with subclinical ketosis and 2 were with clinical ketosis **Tables 1 and 5**.

Health status in control group: The healthy animals are 32 out of 34, 2 were dead and 5 were aborted **Table 6**.

Table 5: BHB Level.

Total Animal	Normal	Subclinical Ketosis	Clinical Ketosis
32	25	5	2
%	78.13%	15.62%	6.25%

Table 6: Health Status in the control group.

Total Animal	Healthy	Dead	Aborted
34	32	2	5
%	94.10%	5.90%	14.70%

Table 7: BHB/Glucose Measurements, test group.

Before Lambing					After Lambing				
Animal No	Date	BHB mmol/l	Glucose mg/dl	Date	BHB mmol/l	Glucose mg/dl	BHB reading difference	Glucose reading Difference	Comments
37858	28-Jul	1	78	24-Aug	0.9	55	Improved	Decreased	
41259	28-Jul	2.1	43	24-Aug	0.6	75	Improved	Increased	Hyperglycaemic
36995	28-Jul	0.8	46	24-Aug	0.8	61	No Change	Improved	
50977	28-Jul	0.9	42	24-Aug	0.6	62	Improved	Improved	
37278	28-Jul	0.9	51	24-Aug	0.6	63	Improved	Improved	
36072	28-Jul	0.7	47	24-Aug	0.4	37	Improved	Decreased	Hypoglycaemic
52527	28-Jul	0.8	40	24-Aug	0.5	57	Improved	Improved	
30897	28-Jul	0.8	32	24-Aug	0.4	43	Improved	Improved	
51955	28-Jul	0.7	50	24-Aug	0.4	61	Improved	Improved	
50909	28-Jul	0.8	44	24-Aug	1.2	65	Increased	Improved	
41933	28-Jul	0.9	43	24-Aug	0.6	57	Improved	Improved	
50903	28-Jul	1.2	53	24-Aug	1.1	53	Improved	No change	
29744	28-Jul	0.9	49	24-Aug	0.7	69	Improved	Improved	
29726	28-Jul	0.8	86	24-Aug	0.5	58	Improved	Decreased	
19370	28-Jul	0.8	51	24-Aug	0.7	58	Improved	Improved	
39470	28-Jul	1.7	69	24-Aug	0.5	61	Improved	Decreased	
51949	28-Jul	1.2	50	24-Aug	0.7	63	Improved	Improved	
36246	28-Jul	0.9	55	24-Aug	0.6	79	Improved	Increased	Hyperglycaemic
30065	28-Jul	1.5	84	24-Aug	2.3	54	Increased	Decreased	
30160	28-Jul	0.8	56	24-Aug	0.6	63	Improved	Increased	
30218	28-Jul	1.1	47	24-Aug	0.7	43	Improved	Decreased	
41295	28-Jul	1.3	51	24-Aug	1.3	83	No change	Increased	Hyperglycaemic
30103	28-Jul	1	84	24-Aug	0.5	66	Improved	Decreased	
52545	28-Jul	1.2	61	24-Aug	0.6	64	Improved	Increased	
52548	28-Jul	0.9	58	24-Aug	0.5	61	Improved	Increased	
29918	28-Jul	0.7	49	24-Aug	0.6	61	Improved	Increased	
36587	28-Jul	1.1	40	24-Aug	0.8	69	Improved	Increased	
40064	28-Jul	0.9	55	24-Aug	0.6	73	Improved	Increased	Hyperglycaemic
36425	28-Jul	0.7	62	24-Aug	1.1	81	Increased	Increased	Hyperglycaemic
34354	28-Jul	0.7	61	24-Aug	0.4	40	Improved	Decreased	
52526	28-Jul	0.9	61	24-Aug	1.7	41	Increased	Decreased	
19861	28-Jul	0.7	56	24-Aug	0.7	57	No change	Increased	
36844	28-Jul	0.7	64	24-Aug	0.8	57	No change	Decreased	
34175	28-Jul	0.7	51	24-Aug	2	34	Increased	Decreased	Hypoglycaemic
34962	28-Jul	1	40	24-Aug	Died	Died	Died	Died	
Normal Range		Normal ≤ 0.8 mmol/l	Normal 40-65 mg/dl		Normal ≤ 0.8 mmol/l	Normal 40-65 mg/dl, Hypoglycaemic, <40 mg/dl			
		Subclinical >0.8 mmol/l	Hypoglycaemic, <40 mg/dl		Subclinical > 0.8 mmol/l	<Hyperglycaemic, >70 mg/dl			
		Clinical > 3 mmol/l	Hyperglycemic, > 70 mg/dl		Clinical > 3 mmol/l				

Pre-lambing period results in the test group

Glucose results: Animal with normal glucose level is 30 out of 35, Hypoglycemic is 1 and hyperglycemic are 4 **Tables 7 and 8**.

Biochemical results and health status pre-lambing summary (Test Group).

BHB results: The animal with normal BHB are 15 out of 35 animals, 20 are with subclinical ketosis and 0 with clinical ketosis **Tables 7 and 9**.

After lambing period results in the test group

Glucose results: Animals with normal glucose level are 27, 2 are hypoglycemic and 5 are hyperglycemic **Tables 7 and 10**.

Biochemical status and health status after Lambing summary (Test Group).

BHB level: 25 animals are with normal BHB, 5 are with subclinical ketosis and 2 are with clinical ketosis **Tables 1 and 11**.

Health status in control group: The healthy animals are 32 out of 34, 2 were dead and 5 were aborted **Table 6**.

Heath status in test group: The healthy animal was 34 out of 35, 1 was dead and 1 was aborted **Table 12**.

Table 8: Glucose level.

Total Animal	Normal	Hypoglycemic	Hyperglycemic
35	30	1	4
%	85.70%	2.90%	11.40%

Table 9: BHB Level.

Total Animal	Normal	Subclinical Ketosis	Clinical Ketosis
35	15	20	0
%	42.86%	57.14	0

Table 10: Glucose Level.

Total Animal	Normal	Hypoglycemic	Hyperglycemic
34	27	2	5
%	79.40%	5.90%	14.70%

Table 11: BHB Level.

Total Animal	Normal	Subclinical Ketosis	Clinical Ketosis
34	26	8	0
%	76.50%	23.50%	0%

Table 12: Health Status in the test group.

Total Animal	Healthy	Dead	Aborted
35	34	1	1
%	97.14%	5.86%	2.86%

Discussion

Ketosis occurs when sheep metabolize body fat to meet their bodies energy need of postpartum milk production. Metabolism of body fat results in an increased production of Beta-hydroxybutyrate (BHB). And other ketone bodies that can be detected in body fluids and milk. It is also very important to know whether your sheep is hypo-, normo-, or hyperglycemic state. Glucose levels can affect sheep metabolism and health. Furthermore, glucose can be read alongside with BHB level to determine if a sheep is in a state of subclinical or clinical ketosis. The gold standard test for subclinical ketosis is blood Beta-hydroxybutyrate (BHB) which is more stable ketone body than acetone or acetoacetate BHB Check Plus (Blood Ketone and Glucose test system) from PortaCheck, USA and also Qucare Vet Meter with Qucare vet strips from DFI, South Korea are semi-quantitative tests and they are intended solely as on farm screening test as they are very simple and rapid. Both systems were used in monitoring BHB and glucose levels in the control and test group. The results of this study also showed that there is a weak correlation between BHB and glucose in subclinical ketotic sheep and blood glucose levels are not a precise and reliable index for evaluation of SCK in sheep herd.

At the time of calving and during negative energy balance, feeding ReaShure increases fat export out of the liver which prevents fatty liver and reduces the amount of fatty acids converted to ketone by the liver and reduces excessive ketone production.

In this study, the direct measurement of BHB in the test group which was fed ReaShure indicated a reduction in subclinical ketosis. It also improves BHB level in 14 sheep >0.8 mmol/l to normal BHB level <0.8 mmol/l.

Conclusion

In conclusion, the impact of feeding of ReaShure to sheep is obvious in reducing in incidence of ketosis in sheep (pregnancy toxemia). Sheep fed ReaShure are healthier compared to those not fed ReaShure. Also, it reduced the incidence of abortion.

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